España; [4] *Unidad de Investigación, Hospital Universitario Santa Cristina, Instituto de Investigación Sanitaria Princesa (IIS-IP), Madrid, España*

* Corresponding author

Fibrinogen is an abundant blood plasma glycoprotein formed by two hetero-trimers composed of the chains Aα, Bβ and γ. It plays an important role in blood clotting (fibrin formation and fibrinolysis), cellular and matrix interactions, inflammatory response, wound healing, and neoplasia[1]. These functions are regulated by interactive sites within the molecule, and when those sites are altered functional impairments arise[2]. Nitroxidative posttranslational modifications occur during nitroxidative distress, when the production of oxygen and nitrogen reactive species surplus the antioxidant capacity, with harmful effects on biomolecules as proteins, nucleic acids and lipids[3,4]. The addition of a nitro functional group in the ring of tyrosine residue producing 3nitrotyrosine is a marker of nitroxidative distress reported present in cardiovascular and inflammatory diseases. The nitration of fibrinogen is present in thrombotic diseases as thrombosis, myocardial infarction and stroke[5,6], and alterations in fibrin polymerization, fibrinolysis and cellular interactions are observed when 3nitrotyrosine is present[7,8,9]. The objective of the present work is to explore the possible functional impact of nitration of fibrinogen as a diagnostic tool for cardiovascular diseases as ischemic stroke.

Enriched fibrinogen from human plasma was *in vitro* nitrated with peroxynitrite and, after in-gel protein digestion, reverse phase-liquid chromatography tandem mass spectrometry (dynamic exclusion mode) was performed. The data processing was carried out with PEAKS Studio XPro software for protein identification and PTM characterization. Finally, fibrinogen visualization and addition of observed 3-nitrotyrosine residues was made in Molecular Operating Environment software.

Sixteen of the 52 tyrosine residues were found to be nitrated within the fibrinogen molecule, being 3 in the Aα chain, 9 in the Bβ chain and 4 in the γ chain. Nine of the 3-nitrotyrosine positions found were within important sites which play roles in mediating fibrin assembly, interaction with thrombin, tissue plasminogen activator, plasminogen and α2-plasmin inhibitor (all of them regulators of coagulation processes). They were in sites related with integrin binding to cells, including leukocytes, platelet spreading, fibroblast proliferation, endothelial cell spreading, proliferation and capillary tube formation, release of von Willebrand factor and binding heparin. It results of interest to investigate the impact of nitration of tyrosine residues on fibrinogen structure and properties in order to shed light into the subjacent mechanisms of diseases with high incidence and mortality rates as thrombotic ones.

doi: https://doi.org/10.1016/j.freeradbiomed.2023.03.258

PP XV_L2

THE ROLE OF PEROXIPORINS IN TUNING THE TRANSMISSION OF INTRACELLULAR REDOX SIGNALING

Ilaria Sorrentino [1], Angie Molina-Oviedo [1], Eduardo Arevalo-Nuñez de Arenas [1], Mauro Galli [1], Stefano Bestetti [2], Roberto Sitia [2], Iria Medraño-Fernandez [1].
[1] *Redox Signaling in Regenerative Medicine lab, Tissue Engineering and Regenerative Medicine (TERMeG), Dept. of Bioengineering, University Carlos III of Madrid, Madrid, Spain;* [2] *Protein transport and secretion Unit, Division of genetic and cell biology, Vita-Salute San Raffaele University, Milan, Italy*

For many years H2O2 has been considered a waste in cells, a relic of when oxygen appeared. Nowadays, this concept is completely reverted, and it is widely accepted that H_2O2 acts as a signaling molecule capable of amplifying different intracellular pathways1,2. Interestingly, H2O2-sources are localized behind double membranes. Hence, it is not surprising that specific proteinaceous channels are responsible for tightly regulating the fluxes of H2O2 across biological membranes. In the past, we characterized aquaporin- 8 (AQP8) as a main H2O2 transporter at the plasma membrane, allowing for the transmission of fluxes after Tyrosine Kinase Receptor (TKR) activation. Moreover, we found that AQP8 can be regulated from an open to a closed conformation following cellular needs3. During the last years, we have also demonstrated that the endoplasmic reticulum (ER) accommodates a specific H2O2 transporter, AQP11, that conducts a constitutive H2O2-flux from the ER to the cytosol that maintains cellular redoxstasis4. To depict the nature of this signal, we have used stable cell lines equipped with the H2O2-sensitive biosensor HyPer targeted to different subcellular compartments. We have discovered that AQP11 can also transmit an H2O2-flux that different strategies, we have determined that the silencing of this protein triggers the activation of ROS production by the mitochondrial complex III5. Thus, we postulate that the existence and the architecture of these different redox fluxes are fundamental to achieving redox homeostasis.

doi: https://doi.org/10.1016/j.freeradbiomed.2023.03.259

PP XIII_L4

EFFECT OF RONS IN 6-NBDG/GLUCOSE UPTAKE IN C2C12 MYOTUBES AND SINGLE ISOLATED SKELETAL MUSCLE FIBRES

Eva Martín-Prieto [1,2,*], Escarlata Fernández-Puente [1,2,3], Jesús Palomero [1,2,3].
[1] *Department of Physiology and Pharmacology, University of Salamanca, Spain;* [2] *Institute of Neurosciences of Castilla y León (INCYL), Spain;* [3] *Institute of Biomedical Research of Salamanca (IBSAL), Spain*

* Corresponding author

Oxidative eustress, which refers to moderate intracellular level of Reactive Oxygen and Nitrogen Species (RONS), is essential to modulate redox signalling processes that maintain and govern cellular homeostasis and adaptation. In skeletal muscle, glucose uptake is essential to provide to the cell the substrate to produce energy through ATP, which is needed as the energy molecule by this organ and others to maintain vital functions. It has been reported that the homeostasis and level of RONS may modulate glucose uptake in skeletal muscle. Hydrogen peroxide (H2O2) and nitric oxide (NO) are two specific RONS that are responsible for oxidative eustress, and they act as signalling molecules that interact with components of the cellular signalling pathways that modulate and regulate glucose uptake in skeletal muscle. We have adapted, developed, optimized, and validated a methodology based on the fluorescence glucose analogue 6-NBDG, combined with quantitative fluorescence microscopy image analysis, to determine the glucose uptake in two models of skeletal muscle cells: C2C12 myotubes and single fibres isolated from muscle. We have assayed different experimental conditions that affect redox homeostasis, such as exposure of cells to different concentrations of H2O2, pro-oxidant environment, impairment, and replenishment of the main antioxidant -glutathione-, and exposure of cells to NO donors. The results showed that pro-oxidative intracellular redox environment evoked by RONS, such as H2O2 and NO, and which might be associated with oxidative eustress, improved 6-NBDG/glucose uptake in myotubes and skeletal muscle fibres. However, when oxidation was excessive (i.e., oxidative distress), cells appeared damaged and cellular viability was compromised. Moreover, in this circumstance, glucose uptake appeared to be elevated. In conclusion, the determination of 6-NBDG/glucose uptake in myotubes and skeletal muscle cells is feasible and was validated. Furthermore, a pro-oxidative environment may favour glucose uptake in C2C12 myotubes and skeletal muscle fibres.

doi: https://doi.org/10.1016/j.freeradbiomed.2023.03.260